Polychlorinated Biphenyl Interactions with Tissue Culture Cells†

by Roger Hoopingarner, Albert Samuel, and David Krause*

The successful use of tissue culture cells in biological work is now almost 60 years old. However, the applicability or the "fitness" of these cells to demonstrate biological principals is still debated. It is well known that many of the cell lines bear little resemblance to the parent tissue or organ cells. Yet isolated functional cells of serially-propagated clonal strains, that can perform specialized, organ-specific functions for prolonged periods, have been produced in nearly limitless numbers (1). The loss of specific function has been ascribed to (i) selective overgrowth by connective tissue cells, (ii) a phenotypic change in the cultured cells, or (iii) inadequate or harmful environmental conditions (2). In order to circumvent these problems, primary cell lines are used and tested before the line "adapts" to culture conditions through aneuploidy or other means.

The use of tissue culture cells for the examination of environmental chemicals has been relatively recent (3). The effects measured have been (i) inhibitory dose, (ii) toxic dose, (iii) cytotoxic effects, and (iv) chromosome aberrations. Samuel (4) has used both established and primary cell lines also to study the metabolism of certain pesticides. Litterst and Lichtenstein (3) using HeLa cells and a cell line of non-malignant origin found that the interaction of DDT and PCBs (Aroclor 1254) that had been found in flies was absent. They concluded that the various interactions of chemicals with the cell lines had no correlation to intact animals.

The use of human primary cell lines was enhanced greatly with the discovery of the mitotic stimulus of the mucoprotein phytohaemagglutinin (5). The leucocyte culture techniques developed by Moorehead et al. (6) proved to be most suitable for in vitro cell studies of human cells and chromosomes. The method also allows an in vivo check for chromosome effects (7) and, as such, may offer a comparison of in vivo with in vitro effects on essentially the same cell.

In the studies to be reported here, we used Chinese hamster cells (quasi-diploid epithelial cells, CH-461) and primary human lymphocyte cells. The Chinese hamster cell line has a generation time of approximately 24 hours. They were grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum.

The lymphocyte cultures were obtained from blood of laboratory volunteers and were grown in chromosome medium 1A from Grand Island Biological Company. The cell cycle time varies with individual donors but is approximately 24 hours after a "lag" period of from 24 to 48 hours prior to activation.

The PCBs were obtained from the Monsanto Company and were used without alteration. The gas chromatography of the PCBs was accomplished using a 50 foot ×0.02 inch S.C.O.T. column of Apienzon L at 200°C. Detection was by hydrogen flame ionization detector.

The growth suppression of Chinese hamster cells with PCBs and DDT are shown in Fig. 1. These data are comparable to those found by Litterst and Lichtenstein (3) though they did not directly study an ID_{50} using both chemicals

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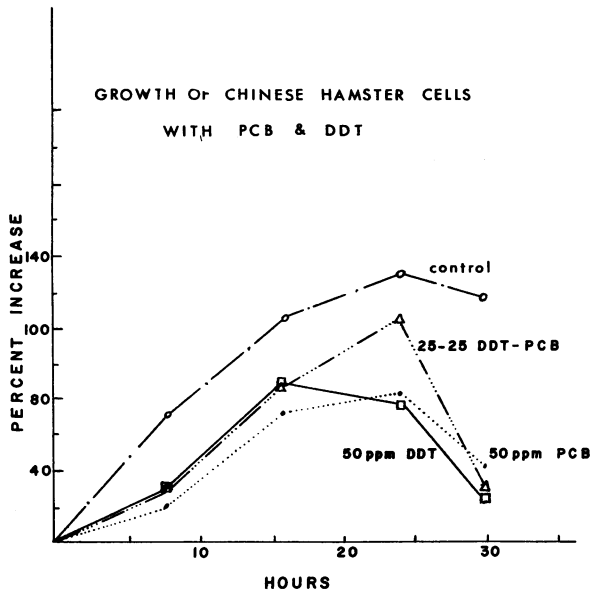

FIGURE 1. Growth of Chinees Hamster cells with PCB and DDT.

simultaneously. The toxic dose of the chemical was several times greater than is usually found biologically.

It is of some interest to note the disintegration of cells in the period from 24 to 30 hours, when the cells were being stressed from culture conditions. This same phenomenon can be seen from the differences between 24 and 30 hours in Table 1. This Table gives the relative growth information for the different PCBs. As you can see, there was a steady drop in cell numbers as one decreases the percentage of chlorine. The Aroclor 1016 is a distillation product of 1254, which essentially excludes the biphenyls of five chlorines

Table 1. Populations of Chinese Hamster Cells Cultured with 50 ppm of Various PCB's

PCB	24 hrs*	30 hrs
None	35±5.9	36±4.4
1260	27±5.6	7±1.7
1254	23±5.8	9±1.7
1248	18±6.1	5±1.4
1221	16±6.4	11±3.4
1016	4±1.1	1±0.4

* Average of ten replicates. Count of cells/unit ocular grid area.

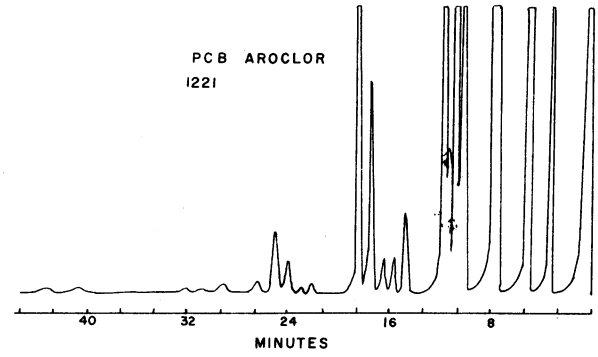

FIGURE 2. Gas Chromatogram of Aroclor 1221.

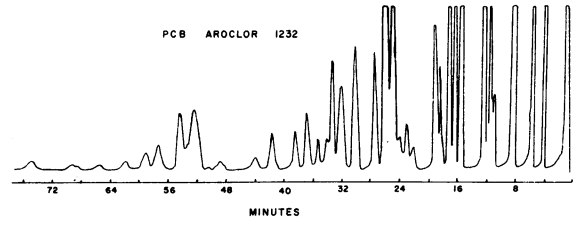

FIGURE 3. Gas Chromatogram of Aroclor 1232.

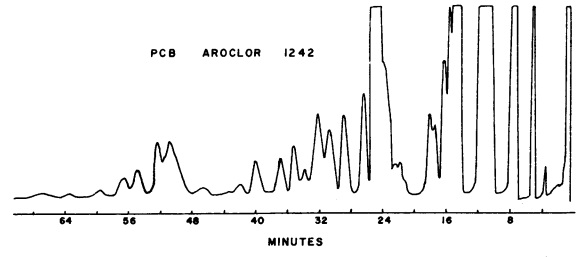

FIGURE 4. Gas Chromatogram of Aroclor 1242.

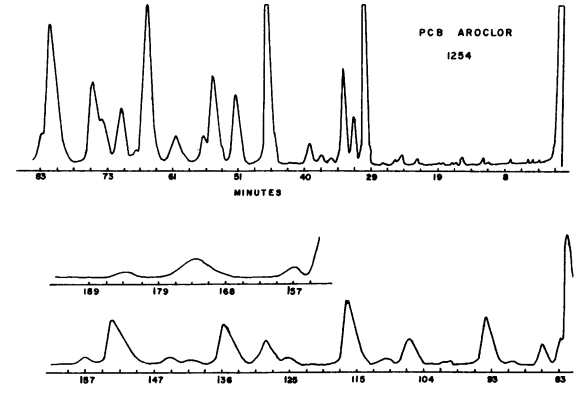

FIGURE 5. Gas Chromatogram of Aroclor 1254.

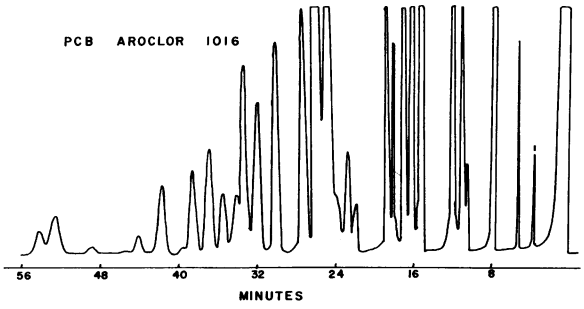

FIGURE 6. Gas Chromatogram of Aroclor 1016.

and above. The product is still a biphenyl, but since it is derived differently, the prefix is changed, from 12 to 10. Based on the percent chlorine, Aroclor 1016 falls out of the pattern of toxicity. If you look at the gas chromatographic patterns, this toxicity is somewhat of an anomaly. For example, the Figures 2 through 6 are the chromatograms for the various PCBs using the support-coated open tubular, or S.C.O.T., columns.

The pattern of 1016 was very much like that found for 1232 or 1242 yet 1016 was several fold more toxic to the tissue culture cells. It would appear that there may be certain components that are in higher proportion in the 1016 product, and these components (or component) are causing the increase in toxicity. We have fractionated most of the chromatographic peaks into their component parts and are currently assaying them to determine the relative toxicity of each.

The human lymphocyte cultures are of course a primary cell line, and in fact we examine the cells cytologically at the first in vitro division.

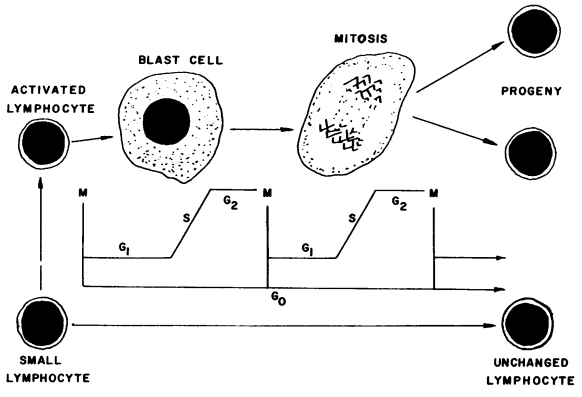

FIGURE 7. Diagram of Cultured human lymphocytes at first in vitro division.

Table 2. Human Lymphocytes Treated with 100 ppm Aroclpr 1254.*

Period of PCB Treatment	Mitotic Index	Satellite Association	Chromatid Gap	Chromatid Breaks
Test 1				
0–24 hrs.	.032	9	5	1
44–52 hrs.	.009	15	3	1
49–52 hrs.	.017	3	4	0
Acetone check	.022	6	3	0
Control	.012	4	5	1
Test 2				
0–24 hrs.	.017	10	1	0
44–52 hrs.	.007	4	4	0
49–52 hrs.	.012	4	2	1
Acetone check	.009	4	3	0
Control	.008	8	2	1

* 50 cells analyzed/test/treatment

The cells are shown diagramatically in Fig. 7. Within the body, the lymphocyte is in a differentiated state G_0. These cells are then activated or transformed into a division cycle with phytohemagglutinin. Sasaki and Norman (8) have shown that the small lymphocyte finishes its transformation in 24 to 48 hours after stimulation and begins a period of cell proliferation with a generation time of 22 hours; G_1, S, G_2, and mitosis are 6, 11, 3, and 2 hours, respectively. We treated these cells with Aroclor 1254 at 100 ppm for the first 24 hours and again during the last eight (S plus G_2), and the last three hours (mitosis). During the first 24 hours, the cells would be in some stage between G_0 and G_1. The cells were then examined cytologically for chromosome aberrations for the various stage treatments. The results are shown in Table 2. PCBs seem to have no effect on chromosomes under these conditions.

The results of this study would indicate that established tissue culture lines are more sensitive than primary cultures to at least one PCB mixture. The various PCB mixtures are generally more toxic as one decreases the percentage of chlorine. The distillation product 1016, however, was more toxic to these cells than was indicated from the percent chlorine content or from its gas chromatographic pattern. At 100 ppm of

1254 to human lymphocyte cultures, there was no apparent effect to the chromosome integrity as measured by cytological evidence.

Tissue culture systems may not always be indicative of whole body conditions. However, these systems do offer some uniform conditions for many definitive studies of environmental chemicals. With substantial data for comparative tests they may be good indicator systems. The human lymphocytes system also offers the comparative cytological test for both in vivo or in vitro exposure.

REFERENCES

1. Yasumura, Y., Tashjian, A. H. and Sato, G. H. 1966. Establishment of four functional, clonal strains of animal cells in culture. Science 154: 1186.
2. Eagle, H. 1965. Metabolic controls in cultured mammalian cells. Science 148: 142.

3a. Gabliks, J. 1965. Response of cell cultures to insecticides. II. Chronic toxicity and induced resistance. Proc. Soc. Exp. Biol. Med. 120: 168.

b. Gabliks, J. 1965. III. Altered susceptibility to poliovirus and diphtheria toxin. Proc. Soc. Exp. Biol. Med. 120: 172.

c. Gabliks, J. and Friedman, L. 1965. Responses of cell cultures to insecticides. I. Acute toxicity to human cells. Proc. Soc. Exp. Biol. Med. 120: 163.

d. Litterst, C. L. and Lichtenstein, E. P. 1971. Effects and interactions of environmental chemicals on human cells in tissue culture. Arch. Environ. Health 22: 454.

e. Hoopingarner, R. and Bloomer, A. W. 1970. Lymphocyte chromosome analysis of pesticide exposed individuals. VII. Int. Cong. Plant Protection. Paris.

4. Samuel, A. 1971. Effect of malathion on selected insect and mammalian tissue cultures. Ph.D. Thesis, Michigan State University.
5. Nowell, P. C. 1960. Phytohaemagglutinin: an initiator of mitosis in cultures of normal human leucocytes. Cancer Res. 20: 462.
6. Moorehead, P. S. et al. 1960. Chromosome preparations of leucocytes cultured from human peripheral blood. Exp. Cell Res. 20: 613.
7. Krause, D. H. 1970. The cytological effects of pesticides on human chromosomes. M.S. Thesis, Michigan State University.
8. Sasaki, M. S. and Norman, A. 1966. Proliferation of human lymphocytes in culture. Nature 210: 502.